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SO1 *Molecular basis for the nitric oxide sensitivity of a blue fluorescent protein*

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Genetically encoded fluorescent sensors for the visualization of nitric oxide (NO) at the singe cell level are still poorly developed. [1] We have recently reported the NO sensitivity of the blue-emitting fluorescent protein mTagBFP2. Protein mutants and mass spectrometry suggested that S-nitrosylation of Cys residues is at the basis of the observed reduction in emission intensity and lifetime in response to NO exposure. The potential of this GES for monitoring intracellular NO was shown on HeLa cells transiently expressing mTagBFP2. [2] In this work, we further investigate the molecular basis for the observed NO sensitivity.
From the experimental point of view, we have performed a more accurate study on the dynamics of S-nitrosylation of Cys residues in mTagBFP2, using the NO donor MAHMA nonoate. The resulting changes in fluorescence emission intensity and lifetime were monitored as a function of time to retrieve a better estimate of the dissociation constant and of the reaction kinetics. We have also tested the response of mTagBFP2 to the presence of the NO donors S-nitrosoglutathione (GSNO) and S-nitroso-N-acetylpenicillamine (SNAP). Fluorescence quenching and mass spectrometry experiments on mTagBFP2 mutants containing only one of the Cys residue show that two out of the three Cys residues in mTagBFP2 are responsible for the changes in fluorescence emission. Further experiments on bacterial cells expressing mTagBFP2 and its mutants, are underway. We have also investigated possible mechanisms of fluorescence loss upon S-nitrosylation including perturbations in the protein structure, leading to increased chromophore flexibility and subsequent enhancement of non-radiative decay. Additionally, Förster resonance energy transfer (FRET) to the newly acquired NO group(s) is considered, as their absorption spectrum, characterized by n → π* and π → π* bands peaking at 545 and 335 nm respectively, partially overlaps with the emission spectrum of mTagBFP2. Through molecular dynamics simulations, we address the structural alterations upon NO binding and calculate the geometric factors necessary to accurately estimate FRET efficiency. Finally, we discuss how our theoretical and computational findings align with experimental measurements on mTagBFP2 and other FP variants.

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References

[1] Eroglu, E.; Charoensin, S.; Bischof, H.; Ramadani, J.; Gottschalk, B.; Depaoli, M. R.; Waldeck-Weiermair, M.; Graier, W. F.; Malli, R., Genetic biosensors for imaging nitric oxide in single cells. Free Radic. Biol. Med. 2018, 128, 50-58.
[2] Montali, C.; Abbruzzetti, S.; Franzen, A.; Casini, G.; Bruno, S.; Delcanale, P.; Burgstaller, S.; Ramadani-Muja, J.; Malli, R.; Gensch, T.; Viappiani, C., Nitric Oxide Sensing by a Blue Fluorescent Protein. Antioxidants 2022, 11, (11), 2229